TENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
6013-76PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00621	25/05/2000	28/05/1999
International Patent Classification (IPC) or national classification and IPC A23L3/015		
Applicant UNIVERSITÉ LAVAL et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
12/12/2000	13.09.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Meyer, J-P Telephone No. +49 89 2399 8649

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00621

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-4,6,7,9-17	as originally filed			
5,5a,8,18	as received on	26/06/2001	with letter of	26/06/2001

Claims, No.:

1-7	as received on	26/06/2001	with letter of	26/06/2001

Drawings, sheets:

1/7-7/7	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
   see separate sheet

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-7
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-7
Industrial applicability (IA)	Yes:	Claims	1-7
	No:	Claims	

2. Citations and explanations
   see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

R Item I

The expression "at least two times" introduced into claim 1 is not to be found in the application as filed. This claim contains therefore subject-matter which extends beyond the content of the application as filed.

Re Item V

1) Reference is made to the following documents:

D1: US 5 232 726
D2: US 5 622 678
D3: US 5 788 934.

2) D1 discloses a process for continuously homogenizing and reducing microbial activity comprising pressurizing a liquid food product and passing it through a pressurizing circulating system under a pressure of about 104 MPa (15000 psi) (according to col. 2 and 3 of D1 the known system appears to correspond to a dynamic high pressure homogenizer) while maintaining good flavor and palatability of the liquid food product. During the process the product is warmed up to about 25 °C. A product having a significant reduction in microbial activity is then collected.

3) The subject-matter of claims 1 to 7 being comprised within the disclosures of D1, these claims are not novel (Article 33(2) PCT).

It should be noted that any argument given in favour of novelty and/or inventive step should be refelected in the wording of the claims.

4) It should be noted that it is known from D2 and D3 that pressures of from 50 to 1000 MPa, respectively from 500 to 1000 MPa destroy microorganisms at room temperature.

D3 teaches further that the cycle is repeated until all the product has been sterilized; this feature is described as providing the same advantages as in the present application.
The skilled person would therefore regard it as a normal design option to include this feature in the process described in document D1 in order to solve the problem posed.

The solution proposed in the claims of the present application cannot be considered as involving an inventive step (Article 33(3) PCT).

Re Item VII

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D2 and D3 is not mentioned in the description, nor are these documents identified therein.

Re Item VIII

The comments on D1 at p. 5 of the description according ot which a pressure of 15 000 psi (104 MPa) is considered as a low pressure is misleading as, according to the present application, a pressure of 50 MPa (see claim 2 in connection with claim 1) is claimed as being a high pressure.

it is recognized as discussed in the Nahra patent that physical agitation of milk may also affect the ultimate flavor of the treated product and disturbance of the free falling films will result in such agitation.

US Patent 6,019,947 discloses a method and apparatus for sterilization of a continuous flow of liquid, which utilize hydrodynamic cavitation. This apparatus uses relatively low pressure (200 to 500 PSI), and the only one cellular lytic mechanism is cavitation. The maximum sterilization yield allows reduction in bacterial counts of only 4 logs.

US Patent 5,232,726 discloses a method for reducing the microbial activity in juices by continuous high-pressure homogenization of citric juices. While results in applying this method are highly variable and inconsistent, lower pressure seems to give as much good effects than higher pressure. The maximum pressure of 15 000 psi has been used in this method, which is considered as a low pressure for those well skilled in the art.

Another problem associated with many of the prior art approaches to steam infusion of liquid products is that the devices are not easily cleaned for example with the use of clean-in-place systems. The more internal components in which the product may collect or burn-on, the more difficult the cleaning process.

It would be highly desirable to be provided with a new process allowing pasteurization of liquid food products without affecting the nutritive value, and preserving all other characteristics of the liquid, like flavor.

SUMMARY OF THE INVENTION

One aim of the present invention is to provide a process for continuously reducing presence of microorganisms in liquid food product without denaturation consisting of: a) pressurizing a liquid food product; b) passing a liquid food product to be treated through a

continuous pressurizing circulating system at a non-denaturing temperature comprising a dynamic high pressure homogenizer; and c) collecting the liquid food product containing a reduced presence of microbes.

and collision on the stationary surface, which combine to reduce the size of fat globules.

In a preferred embodiment of the invention, microorganisms are disrupted by a multiplicity of mechanisms during submitting to DHP: the sudden pressure drop, shear stresses, cavitation and impingement. The overall pressure drop and the rate at which it occurs can is responsible for the cell disruption.

It will be apparent to those skilled in the field that the method and apparatus thus described is extremely simple, avoids the problem of product burn-on.

In a particular embodiment of the invention, there is provided with a process to treated liquid food products contaminated, or potentially contaminated with, but not limitatively, Gram positive or Gram negative bacteria, yeast, viruses, protozoan, and mould.

In one embodiment of the invention is to preformed sterilization to pressure up to 40 000 psi(277 Mpa).

In accordance with another embodiment of the invention, the DHP can be applied in inactivating bacteriophages in different liquid food products, or also to inactivate enteric viruses such as Hepatitis A, rotavirus, and Norwalk virus contained in water.

It is recognized from the present invention that several food products lend themselves to preservation by the use of DHP to sterilize the products. DHP sterilization destroys microorganisms and inactivates most enzymes that cause product spoilage.

One embodiment of the invention as extending normal shelf life of fresh food while at same time maintaining nutritional quality and ensuring safety, as for example milk, and cheese.

Finally, Fig. 7 shows the industrial trial compared to laboratory results for *Listeria innocua* under the same treatment conditions as above. A similar reduction was obtained (□:1 pass; ■ :3 passes; ■ :5 passes).

This study has shown the effectiveness of DHP for destroying pathogenic flora in milk. It has been shown to be a viable alternative to conventional milk pasteurisation. A better bactericidal effect was obtained compared to hydrostatic pressure and milk characteristics were not affected. This new technology should be given serious consideration in the milk industry.

The embodiment(s) of the invention described above is(are) intended to be exemplary only. The scope of the invention is therefore intended to be limited solely by the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A process for continuously reducing presence of microorganisms in liquid food product without denaturation comprising the steps of:

a) pressurizing a liquid food product;

b) passing said liquid food product to be treated at least two times through a continuous pressurizing circulating system at a non-denaturing temperature comprising a dynamic high pressure homogenizer; and

c) collecting said liquid food product containing a reduced presence of microbes.

2. The process according to claim 1, wherein said pressure of step a) is between about 50 MPa to 500 MPa.

3. The process according to claim 1, wherein said passage of step b) is at least one passage of said liquid food product through the dynamic high pressure homogenizer.

4. The process according to claim 1, wherein said microorganisms are selected from the group consisting of bacteria, fungi, mould, bacteriophage, protozoan, and virus.

5. The process according to claim 1, wherein said temperature is between about 4°C to 55°C.

6. The process according to claim 1, wherein said homogenizer is a high-pressure homogenizer.

7. The process according to claim 1, wherein said liquid food product is selected from the group consisting of milk, juice, liquid food fat, oil, and water.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
6013-76PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/CA 00/ 00621	25/05/2000	28/05/1999
Applicant		
UNIVERSIT LAVAL et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
P[redacted]A 00/00621

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L3/015

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L A23C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
PAJ, EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 788 934 A (LHENRY BERNARD ET AL) 4 August 1998 (1998-08-04) the whole document ---	1-5,7
X	US 5 232 726 A (CLARK ALLEN V ET AL) 3 August 1993 (1993-08-03) column 2, line 67 -column 3, line 15; claims; figures ---	1-7
X	US 5 622 678 A (HILTAWSKY JOSEF ET AL) 22 April 1997 (1997-04-22) column 4, line 27 - line 33; claims; figures column 1, line 7 - line 24 column 1, line 60 - line 67 --- -/--	1-7

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 September 2000	04/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Guyon, R

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
P[redacted]CA 00/00621

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 411 380 A (DRAINONI ROBERT A ET AL) 2 May 1995 (1995-05-02) the whole document	1-3,5-7
X	DATABASE WPI Section Ch, Week 199528 Derwent Publications Ltd., London, GB; Class D13, AN 1995-207526 XP002147984 & CN 1 086 104 A (WANG Y), 4 May 1994 (1994-05-04) abstract	1
A	US 5 486 372 A (MARTIN ROBERT W ET AL) 23 January 1996 (1996-01-23) column 10, line 31 - line 40; claim 1; examples	1-3,5,7
A	US 5 328 703 A (OCHIAI SHINYA ET AL) 12 July 1994 (1994-07-12) the whole document	1
A	WO 82 02928 A (FOSS ELECTRIC AS N ;PETERSSON MOGENS (DK)) 2 September 1982 (1982-09-02)	
A	PATENT ABSTRACTS OF JAPAN vol. 010, no. 310 (C-379), 22 October 1986 (1986-10-22) & JP 61 119154 A (WORLD FOOD KK), 6 June 1986 (1986-06-06) abstract	
A	EP 0 736 262 A (KRAFT FOODS INC) 9 October 1996 (1996-10-09) the whole document	
A	DE 39 03 648 A (BRAN & LUEBBE ;BAYER AG (DE)) 16 August 1990 (1990-08-16) the whole document	1,3,4
A	PATENT ABSTRACTS OF JAPAN vol. 1997, no. 06, 30 June 1997 (1997-06-30) & JP 09 051784 A (YASUSATO SHIGEO;SHIRANE MASASHI; HIGA MASAO; KUDEKEN KENSHIN), 25 February 1997 (1997-02-25) abstract	

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 00/00621

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 5788934	A	04-08-1998	FR	2730412 A	14-08-1996
			EP	0727227 A	21-08-1996
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			ZA	9305121 A	01-03-1994
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			DE	59506970 D	11-11-1999
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			JP	8054001 A	27-02-1996
US 5411380	A	02-05-1995	NONE		
CN 1086104	A	04-05-1994	NONE		
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			WO	9524132 A	14-09-1995
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			DE	69116070 D	15-02-1996
			DE	69116070 T	19-09-1996
			EP	0480422 A	15-04-1992
WO 8202928	A	02-09-1982	DK	75181 A	20-08-1982
			AU	8144582 A	14-09-1982
			EP	0072835 A	02-03-1983
JP 61119154	A	06-06-1986	JP	1635957 C	31-01-1992
			JP	2059708 B	13-12-1990
EP 0736262	A	09-10-1996	US	5965190 A	12-10-1999
			AT	181800 T	15-07-1999
			DE	69603111 D	12-08-1999
			DE	69603111 T	05-01-2000
			GR	3031350 T	31-01-2000
DE 3903648	A	16-08-1990	DE	3943590 C	24-05-1995
JP 09051784	A	25-02-1997	NONE		

XP-002147984

AN - 1995-207526 [28]
AP - CN19920112401 19921029
CPY - WANG-I
DC - D13
FS - CPI
IC - A23L2/00
IN - DI H; WANG B; WANG Y
MC - D03-H01T2
PA - (WANG-I) WANG Y
PN - CN1086104 A 19940504 DW199528 A23L2/00 000pp
PR - CN19920112401 19921029
XA - C1995-096194
XIC - A23L-002/00
AB - CN1086104 Lotus seeds, whose peels and plumules have been removed are soaked in water, pulped and diluted, then fruit sugar and cyclodextrin. The materials are then homogenised and sterilised under high pressure.
- ADVANTAGE - The syrup is a grey-white uniform cloudy liquor and its taste is fragrant and sweet.
- The syrup nourishes the heart, tones the liver and invigorates the spleen.
IW - LOTUS SEED SYRUP PREPARATION
IKW - LOTUS SEED SYRUP PREPARATION
INW - DI H; WANG B; WANG Y
NC - 001
OPD - 1992-10-29
ORD - 1994-05-04
PAW - (WANG-I) WANG Y
TI - Lotus seed syrup prepn.

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

To:

COTE, France
SWABEY OGILVY RENAULT
1981 McGill College Avenue
Suite 1600
Montréal, Québec H3A 2Y3
CANADA

Date of mailing *(day/month/year)* 13.09.2001

Applicant's or agent's file reference
6013-76PCT

IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00621	25/05/2000	28/05/1999

Applicant
UNIVERSITÉ LAVAL et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer
Lázaro Ortiz, A
Tel.+49 89 2399-8011

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
6013-76PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00621	25/05/2000	28/05/1999
International Patent Classification (IPC) or national classification and IPC A23L3/015		
Applicant UNIVERSITÉ LAVAL et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

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   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

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3. This report contains indications relating to the following items:

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   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
12/12/2000	13.09.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Meyer, J-P Telephone No. +49 89 2399 8649

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00621

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-4,6,7,9-17	as originally filed			
5,5a,8,18	as received on	26/06/2001	with letter of	26/06/2001

Claims, No.:

1-7	as received on	26/06/2001	with letter of	26/06/2001

Drawings, sheets:

1/7-7/7	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under R 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
   see separate sheet

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability: citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-7
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-7
Industrial applicability (IA)	Yes:	Claims	1-7
	No:	Claims	

2. Citations and explanations
   see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item I

The expression "at least two times" introduced into claim 1 is not to be found in the application as filed. This claim contains therefore subject-matter which extends beyond the content of the application as filed.

Re Item V

1) Reference is made to the following documents:

D1: US 5 232 726
D2: US 5 622 678
D3: US 5 788 934.

2) D1 discloses a process for continuously homogenizing and reducing microbial activity comprising pressurizing a liquid food product and passing it through a pressurizing circulating system under a pressure of about 104 MPa (15000 psi) (according to col. 2 and 3 of D1 the known system appears to correspond to a dynamic high pressure homogenizer) while maintaining good flavor and palatability of the liquid food product. During the process the product is warmed up to about 25 °C. A product having a significant reduction in microbial activity is then collected.

3) The subject-matter of claims 1 to 7 being comprised within the disclosures of D1, these claims are not novel (Article 33(2) PCT).

It should be noted that any argument given in favour of novelty and/or inventive step should be refelected in the wording of the claims.

4) It should be noted that it is known from D2 and D3 that pressures of from 50 to 1000 MPa, respectively from 500 to 1000 MPa destroy microorganisms at room temperature.

D3 teaches further that the cycle is repeated until all the product has been sterilized; this feature is described as providing the same advantages as in the present application.
The skilled person would therefore regard it as a normal design option to include this feature in the process described in document D1 in order to solve the problem posed.

The solution proposed in the claims of the present application cannot be considered as involving an inventive step (Article 33(3) PCT).

Re Item VII

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D2 and D3 is not mentioned in the description, nor are these documents identified therein.

Re Item VIII

The comments on D1 at p. 5 of the description according ot which a pressure of 15 000 psi (104 MPa) is considered as a low pressure is misleading as, according to the present application, a pressure of 50 MPa (see claim 2 in connection with claim 1) is claimed as being a high pressure.

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
COTE, France
SWABEY OGILVY RENAULT
Suite 1600
Montréal, Québec H3A 2Y3
1981 McGill College Avenue
CANADA

Date of mailing (day/month/year)	
07 December 2000 (07.12.00)	
Applicant's or agent's file reference 6013-76PCT	**IMPORTANT NOTICE**
International application No. PCT/CA00/00621	**International filing date** (day/month/year) 25 May 2000 (25.05.00)
Priority date (day/month/year) 28 May 1999 (28.05.99)	
Applicant UNIVERSITÉ LAVAL et al	

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AG,AU,DZ,KP,KR,MZ,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CN,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,
   GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,
   NO,NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   07 December 2000 (07.12.00) under No. WO 00/72703

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO 34, ch min des Col mbettes 1211 G neva 20, Switz rland	Authorized officer J. Zahra
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Published:
— *With international search report.*
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/72703 A1

(54) Title: INACTIVATION OF FOOD SPOILAGE AND PATHOGENIC MICROORGANISMS BY DYNAMIC HIGH PRESSURE

(57) Abstract: The present invention relates to a process using dynamic high-pressure for inactivation of food pathogens. Liquid food are treated by dynamic high-pressure at 1 to 5 kbars with at least one recirculation depending on the needs. The pasteurization process is performed at relatively cold temperature ranging from 4 °C to 55 °C.

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L3/015

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L A23C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
PAJ, EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 788 934 A (LHENRY BERNARD ET AL) 4 August 1998 (1998-08-04) the whole document	1-5,7
X	US 5 232 726 A (CLARK ALLEN V ET AL) 3 August 1993 (1993-08-03) column 2, line 67 -column 3, line 15; claims; figures	1-7
X	US 5 622 678 A (HILTAWSKY JOSEF ET AL) 22 April 1997 (1997-04-22) column 4, line 27 - line 33; claims; figures column 1, line 7 - line 24 column 1, line 60 - line 67	1-7

-/—

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 September 2000	04/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Guyon, R

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JP 09051784 A	25-02-1997	NONE	

it is recognized as discussed in the Nahra patent that physical agitation of milk may also affect the ultimate flavor of the treated product and disturbance of the free falling films will result in such agitation.

US Patent 6,019,947 discloses a method and apparatus for sterilization of a continuous flow of liquid, which utilize hydrodynamic cavitation. This apparatus uses relatively low pressure (200 to 500 PSI), and the only one cellular lytic mechanism is cavitation. The maximum sterilization yield allows reduction in bacterial counts of only 4 logs.

Another problem associated with many of the prior art approaches to steam infusion of liquid products is that the devices are not easily cleaned for example with the use of clean-in-place systems. The more internal components in which the product may collect or burn-on, the more difficult the cleaning process.

It would be highly desirable to be provided with a new process allowing pasteurization of liquid food products without affecting the nutritive value, and preserving all other characteristics of the liquid, like flavor.

SUMMARY OF THE INVENTION

One aim of the present invention is to provide a process for continuously reducing presence of microorganisms in liquid food product without denaturation consisting of: a) pressurizing a liquid food product; b) passing a liquid food product to be treated through a continuous pressurizing circulating system at a non-denaturing temperature comprising a dynamic high pressure homogenizer; and c) collecting the liquid food product containing a reduced presence of microbes.

and collision on the stationary surface, which combine to reduce the size of fat globules.

In a preferred embodiment of the invention, microorganisms are disrupted by a multiplicity of mechanisms during submitting to DHP: the sudden pressure drop, shear stresses, cavitation and impingement. The overall pressure drop and the rate at which it occurs can is responsible for the cell disruption.

It will be apparent to those skilled in the field that the method and apparatus thus described is extremely simple, avoids the problem of product burn-on.

In a particular embodiment of the invention, there is provided with a process to treated liquid food products contaminated, or potentially contaminated with, but not limitatively, Gram positive or Gram negative bacteria, yeast, viruses, protozoan, and mould.

In one embodiment of the invention is to preformed sterilization to pressure up to 40 000 psi.

In accordance with another embodiment of the invention, the DHP can be applied in inactivating bacteriophages in different liquid food products, or also to inactivate enteric viruses such as Hepatitis A, rotavirus, and Norwalk virus contained in water.

It is recognized from the present invention that several food products lend themselves to preservation by the use of DHP to sterilize the products. DHP sterilization destroys microorganisms and inactivates most enzymes that cause product spoilage.

One embodiment of the invention as extending normal shelf life of fresh food while at same time maintaining nutritional quality and ensuring safety, as for example milk, and cheese.

Finally, Fig. 7 shows the industrial trial compared to laboratory results for Listeria innocua under the same treatment conditions as above. A similar reduction was obtained (■ :1 pass; ■ :3 passes; □ :5 passes).

This study has shown the effectiveness of DHP for destroying pathogenic flora in milk. It has been shown to be a viable alternative to conventional milk pasteurisation. A better bactericidal effect was obtained compared to hydrostatic pressure and milk characteristics were not affected. This new technology should be given serious consideration in the milk industry.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A process for continuously reducing presence of microorganisms in liquid food product without denaturation comprising the steps of:

a) pressurizing a liquid food product;

b) passing said liquid food product to be treated through a continuous pressurizing circulating system at a non-denaturing temperature comprising a dynamic high pressure homogenizer; and

c) collecting said liquid food product containing a reduced presence of microbes.

2. The process according to claim 1, wherein said pressure of step a) is between about 50 MPa to 500 MPa.

3. The process according to claim 1, wherein said passage of step b) is at least one passage of said liquid food product through the dynamic high pressure homogenizer.

4. The process according to claim 1, wherein said microorganisms are selected from the group consisting of bacteria, fungi, mould, bacteriophage, protozoan, and virus.

5. The process according to claim 1, wherein said temperature is between about 4°C to 55°C.

6. The process according to claim 1, wherein said homogenizer is a high-pressure homogenizer.

7. The process according to claim 1, wherein said liquid food product is selected from the group consisting of milk, juice, liquid food fat, oil, and water.

INACTIVATION OF FOOD SPOILAGE AND PATHOGENIC MICROORGANISMS BY DYNAMIC HIGH PRESSURE

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates to a process for inactivation of contaminating liquid food pathogens, and more particularly to such a process which utilize a dynamic high-pressure treatment.

(b) Description of Prior Art

Every year, outbreaks of illnesses caused by pathogenic bacteria contaminating foods have economic repercussions throughout the world. Due to its composition and mode of production, milk is particularly susceptible to contamination by a wide variety of bacteria. When milk is secreted in the udders of ruminants, it is virtually sterile. Many milk-borne bacteria are casual visitors but find them in an environment where they can live and possibly proliferate. Although some of these bacteria die when competing with species which find the environment more congenial pathogenic bacteria, such as *Listeria, Escherichia, Salmonella,* can survive and create dangers for the consumer.

Heat, for instance pasteurization is still the most commonly used technology to inactivate food spoilage and pathogenic bacteria in raw milk and other liquid foods. Although effective, some bacteria may resist thermal treatment, especially Bacillus and Clostridium. Furthermore, high temperatures may induce undesirable losses of flavor as well as denaturation of certain vitamins and nutritive proteins. Reduction in soluble calcium, formations of complexes between constituents, and reduction of cheese yield have also been observed. For example, thermal decomposition of milk β-lactoglobulin produces volatile sulfur compounds

that may inhibit fermentation, thus affecting the appearance, taste and nutritional value of milk as well as processing characteristics.

In recent years, many alternative methods have been investigated as means of inactivating food spoilage and pathogenic bacteria. Bactofugation and microfiltration have been proposed and shown to reduce the initial microbial load. These processes still required a heat treatment in order to achieve satisfactory results. The advantages of these methods are better microbial quality and longer shelf life. More recently, high hydrostatic pressure (HHP) technology has been proposed as a new strategy to inactivate both the spoilage and pathogenic bacteria. Using this technology, high pressure (5 to 15 kbars or 500 to 1500 MPascal (MPa)) are often needed to achieve the inactivation effect. Such pressures may affect systems determining morphology, biochemical reactions, genetic mechanisms, membrane, and cell wall structure of microorganisms. Sensivity to high pressure varies greatly from one bacterial specy to another. A pressure of 300 MPa (3000 bars) for 10 to 30 minutes is needed for the inactivation of Gram positive bacteria, yeasts and mildew. *Bacillus subtilis* spores are inactivated at 1750 MPa. A pressure of 400 MPa for 20 minutes is required to completely inactivate *E. coli* or bring about an 8-log reduction of *Saccharomyces cerevisiae.* Unfortunately, the principle of this technology is applied as a batch treatment, that is suitable for small volumes, and the establishment of this method on an industrial scale is difficult and costly.

It is well known that ultraviolet light in the proper dose kills most bacteria, algae, viruses, mold spores, and other microorganisms found in liquids such

as water. There have been many ultraviolet water sterilization systems proposed to take advantage of this phenomenon. U.S. Pat. Nos. 4,769,131 and 4,968,437 issued to Noll et al. disclose an ultraviolet purification system in which water is pumped through tubes helically coiled around an ultraviolet lamp to provide maximum ultraviolet exposure time for a given tube length to create a relatively compact sterilization system for potable water.

This system as well as other known systems suffers from a number of drawbacks which make them less than ideal solutions to the water purification problem. Ultraviolet sterilization is not applicable on milk because of the opalescence.

On problem common to these systems is that the liquid must be pumped under pressure past the ultraviolet lamp both before and after filtration. This requires a relatively large pump that draws a relatively great amount of power. In addition, such systems are typically designed to treat tap water, and are incapable of taking water from another source such as collecting water dripping off a condensing coil of a dehumidification or air conditioning system.

In the sterilization of milk, it is necessary to raise the temperature of the milk sufficiently to destroy all bacteria and inactivate enzymes. The rate of destruction or inactivation of these organisms varies_with both temperature and the time during which the product is held at an elevated temperature. A method of sterilizing milk and dairy products has been to utilize steam infusion to subject the milk to ultra high temperatures for very short periods of time followed by flash cooling. This has been proven to achieve superior product flavor. Various approaches have been used in the past to accomplish this. For

example U.S. Pat. No. 3,156,176 to Wakeman describes a heating apparatus in which steam is supplied into a chamber with the liquid product being introduced in the form of a curtain-like film to expose the fluent product to the elevated steam temperatures. Similarly, U.S. Pat. No. 2,899,320 to Davies and U.S. Pat. No. 3,032,423 to Evans, both utilize apparatus for containing steam in which the product is passed over plates within the steam chamber and heated while the product flows downwardly to a collection point for delivery to a flash chamber. A variation of this method is also described in U.S. Pat. No. 3,771,434 to Davies in which screen panels are used to form a thin film of product for exposure to steam. One major disadvantage of the methods and apparatus described in the foregoing patents is the fact that liquid food products, particularly milk products, have a tendency to burn and collect on heated surfaces which are at temperatures greater than or equal to the temperature of the product itself. Such burning, in addition to fouling the apparatus itself necessitating periodic cleaning, also results in undesirable flavor changes to the milk product.

In an obvious effort to avoid such burn-on and fouling, U.S. Pat. No. 4,310,476 to Nahra and U.S. Pat. No. 4,375,185 to Mencacci attempt to form free falling thin films of milk within a steam atmosphere for raising the product temperature. A problem associated with attempting to form a free falling thin film is that the integrity of such films is very unstable and are subject to splashing or break-up in the presence of moving or circulating steam and gases. Film formation requires close adherence to flow parameters and such devices are also subject to the product burn-on problems when hot surfaces are contacted. Additionally,

it is recognized as discussed in the Nahra patent that physical agitation of milk may also affect the ultimate flavor of the treated product and disturbance of the free falling films will result in such agitation.

US Patent 6,019,947 discloses a method and apparatus for sterilization of a continuous flow of liquid, which utilize hydrodynamic cavitation. This apparatus uses relatively low pressure (200 to 500 PSI), and the only one cellular lytic mechanism is cavitation. The maximum sterilization yield allows reduction in bacterial counts of only 4 logs.

Another problem associated with many of the prior art approaches to steam infusion of liquid products is that the devices are not easily cleaned for example with the use of clean-in-place systems. The more internal components in which the product may collect or burn-on, the more difficult the cleaning process.

It would be highly desirable to be provided with a new process allowing pasteurization of liquid food products without affecting the nutritive value, and preserving all other characteristics of the liquid, like flavor.

SUMMARY OF THE INVENTION

One aim of the present invention is to provide a process for continuously reducing presence of microorganisms in liquid food product without denaturation consisting of: a) pressurizing a liquid food product; b) passing a liquid food product to be treated through a continuous pressurizing circulating system at a non-denaturing temperature comprising a dynamic high pressure homogenizer; and c) collecting the liquid food product containing a reduced presence of microbes.

Another aim of the present invention is to provide a process wherein the pressure used is between 50 MPa to 500 MPa.

In accordance with the present invention there is provided also a process that needs at least one passage of the liquid food product through the dynamic high-pressure homogenizer.

Another aim of the present invention is to provide a process wherein the microorganisms to be killed may be selected from bacteria, fungi, mould, bacteriophage, protozoan, and virus.

The process may be performed using a milk homogenizer at temperature between 4°C to 55°C.

Also, one aim of the invention is to provide a process of sterilizing several liquid food products as of milk, juice, liquid food fat, oil, and water.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the inactivation of three major food pathogens in phosphate buffer by DHP as a function of applied pressure (100, 200 and 300 MPa) and the number of passes (1, 3 and 5).

Fig. 2 illustrates the inactivation of *Listeria monocytogeneses* (■), *Salmonella enteritidis* (■), *Escherichia coli* (□) in phosphate buffer by DHP (200 MPa/1 pass) after a mild heat treatment for 10 minutes at 4, 25, 45 or 55 °C.

Fig. 3 illustrates the inactivation of *Listeria monocytogeneses* (▣), *Salmonella enteritidis* (■) and *Escherichia coli* (□) in phosphate buffer by DHP (200 MPa/1 pass) as a function of initial bacterial load (10^4 to 10^9).

Fig. 4 illustrates the inactivation of two major food pathogens in raw milk by DHP as a function of applied pressure (100, 200 and 300 MPa) and number of passes (1, 3 and 5).

Fig. 5 illustrates the inactivation of two major food pathogens in raw milk by DHP (200 MPa/1 pass) in response to a mild heat treatment of 10 minutes (25, 45, 55 and 60 °C).

Fig. 6 illustrates the inactivation of two major food pathogens in raw milk by DHP (200 MPa/1 pass) as a function of initial load (10^5 to 10^8).

Fig. 7 illustrates the inactivation of *Listeria innocua* (10^7 CFU/ml) in raw milk by DHP (200 MPa) at a laboratory (Emulsiflex-C5) or industrial scale (Emilsiflex-C160).

DETAILED DESCRIPTION OF THE INVENTION

The use of dynamic high-pressure to inactivate food pathogens has never been reported. In contrast to hydrostatic high-pressure treatment (HHP), the dynamic high pressure (DHP) uses low pressure, as about 2 kbars to achieve same bacteria inactivation results. At this relatively low pressure, food constituents are better preserved from mechanical and biophysical damages well characterized in other sterilization approaches.

In accordance with the present invention, there is provided an new alternative to liquid food pasteurization, that is to say dynamic high pressure (DHP). In the milk industry, light pressure homogenization is used to reduce the diameter of fat globules in order to prevent creaming. Pressure is applied to a liquid forced through an adjustable valve causing increased flow speed and a pressure loss, bringing about cavitation, chisel effect, turbulence

and collision on the stationary surface, which combine to reduce the size of fat globules.

In a preferred embodiment of the invention, microorganisms are disrupted by a multiplicity of mechanisms during submitting to DHP: the sudden pressure drop, shear stresses, cavitation and impingement. The overall pressure drop and the rate at which it occurs can is responsible for the cell disruption.

It will be apparent to those skilled in the field that the method and apparatus thus described is extremely simple, avoids the problem of product burn-on.

In a particular embodiment of the invention, there is provided with a process to treated liquid food products contaminated, or potentially contaminated with, but not limitatively, Gram positive or Gram negative bacteria, yeast, viruses, protozoan, and mould.

In one embodiment of the invention is to preformed sterilization to pressure up to 40 000 psi.

In accordance with another embodiment of the invention, the DHP can be applied in inactivating bacteriophages in different liquid food products, or also to inactivate enteric viruses such as Hepatitis A, rotavirus, and Norwalk virus contained in water.

It is recognized from the present invention that several food products lend themselves to preservation by the use of DHP to sterilize the products. DHP sterilization destroys microorganisms and inactivates most enzymes that cause product spoilage.

One embodiment of the invention as extending normal shelf life of fresh food while at same time maintaining nutritional quality and ensuring safety, as for example milk, and cheese.

Also, the invention relates to a process for eliminating lactic acid bacteria bacteriophages from cheese plant by treating milk and whey samples.

An another embodiment of the invention is that DHP sterilization of certain food products may eliminate the need for refrigeration. This is particularly true in the case of dairy products such as milk or ice cream mix, to which this invention is primarily directed, although it may be equally applied to other liquid products such as juices.

While the invention has thus been described in relation to a process for treating milk, others skilled in the art will appreciate that other food products in liquid form may also be sterilized as well such as flavored milk, half and half, dairy creams, whipping creams, condensed milk, ice cream milk, shake mix, puddings, custard, fruit juices, etc. Adjustments to the operating pressure and flow rates may be necessary but these variations will be recognized and easily addressed by those skilled in the field.

EXAMPLE 1

INACTIVATION OF SOME FOOD PATHOGENS USING DYNAMIC HIGH PRESSURE

Every year, outbreaks of illnesses caused by pathogenic bacteria contaminating foods have economic repercussions throughout the world. Due to its composition and mode of production, milk is particularly susceptible to contamination by a wide variety of bacteria. When milk is secreted in the udders of ruminents, it is virtually sterile. Many milk-borne bacteria are casual visitors but find themselves in an environment where they can live and

possibly proliferate. Although some of these bacteria die when competing with species which find the environment more congenial pathogenic bacteria such as *Listeria, Escherichia, Salmonella,* etc, can survive in milk and create dangers for the consumer.

Heat (e.g. pasteurisation) for instance pasteurisation is still the most commonly used technology to inactivate food spoilage and pathogenic bacteria in raw milk. Although effective, some bacteria may resist thermal treatment, especially *Bacillus* and *Clostridium*. Furthermore, high temperatures may induce undesirable losses of flavours as well as denaturation of certain vitamins and proteins. Reduction in soluble calcium, formation of complexes between β-lactoglobulin and κ-casein and reduction of cottage cheese yield have also been reported. Thermal decomposition of β-lactoglobulin produces volatile sulfur compounds (Desmazeaud, 1990) which may inhibit lactic fermentation, thus affecting the appearance, taste and nutritional value of milk as well as its processing characteristics.

In recent years, many alternative methods have been investigated as means of inactivating food spoilage and pathogenic bacteria. Bactofugation and microfiltration shows to reduce the initial microbial load. These processes still required a heat treatment in order to achieve satisfactory results. The advantages of these methods were better microbial quality and longer shelf life. Recently, high hydrostatic pressure (HHP) technology has been proposed as a new strategy to inactivate both the spoilage and pathogenic bacteria. Using this technology, high pressures (1 to 15 kbars or 100 to 1 500 MPa) are often

needed to achieve the inactivation effect. Such pressures may affect systems determining morphology, biochemical reactions, genetic mechanisms, membrane and cell wall structure of microorganisms. Sensitivity to high pressure varies greatly from one bacterial species to another. A pressure of 300 MPa (3 000 bars) for 10 to 30 minutes is needed for the inactivation of Gram negative bacteria, yeasts and mildew. *Bacillus subtilis* spores are inactivated at 1 750 MPa (17 500 bars). A pressure of 400 MPa for 20 minutes is required to completely inactivate *E. coli* or bring about an 8-log reduction of *Saccharomyces cerevisiae*. Furthermore, 500 MPa at 25°C for 20 minutes is required to completely inactivate *Listeria innocua*. The principle of this technology is applied as a batch treatment, which is suitable for small volumes but the establishment of this method on an industrial scale is difficult and costly.

Another alternative to heat is dynamic high pressure (DHP). In the milk industry, light pressure homogenization is used to reduce the diameter of fat globules in order to prevent creaming. Pressure is applied to a liquid forced through an adjustable valve causing increased flow speed and a pressure loss, bringing about cavitation, chisel effect, turbulence and collision on the stationary surface, which combine to reduce the size of fat globules. The effects of DHP on bacterial cells are not yet well known. Some studies have shown changes in cell morphology as well as splits in the cytoplasmic membrane. Decreased numbers of ribosomes and the formation of spongy clear areas within the cytoplasm have also been observed. Research has shown that the cellular membrane is the

site most damaged by pressure. Made of phospholipids and proteins held together by hydrogen bonds ties and hydrophobic bonds, the membrane is somewhat rigid and plays a significant role in cellular respiration and transport. Increases in permeability or rupture of the cell membrane, as may happen under pressure, cause cell death. Based on this principle, DHP technology may offer a promising alternative for the cold pasteurization of milk and perhaps other liquid foods by inactivating bacterial contaminants. A more effective inactivation may be achieved using DHP compared to HHP.

The objective of this study is to evaluate the effectiveness of a dynamic high-pressure treatment for the inactivation of three major food pathogens *Listeria monocytogeneses, Salmonella enteritidis* and *Escherichia coli O157:H7* in raw milk.

Material and methods

Sample preparation: Three bacterial strains were used in this study: as *Listeria monocytogenese* (Canadian Food Inspection Agency #105-1) as Gram positive and *Escherichia coli* O157:H7 (ATCC #35150) and *Salmonella enteritidis* (ATCC #13047) as Gram negative representatives. Bacterial strains were maintained as glycerol stock at -80°C. When needed, strains were inoculated in tryptic soy broth (Difco) and incubated at 37°C for 12 to 18 hours. The culture was then centrifuged at 7 000 rpm for 15 minutes, washed 2 times in phosphate buffer and then used to inoculate different samples of raw milk and phosphate buffer. The final bacterial concentration was determined by enumeration on tryptic soy agar (Difco). The

efficiency of the DHP treatment was estimated by the enumeration of residual bacteria in the sample and was expressed as N/N_o when N_o is the bacterial count before the DHP treatment and N, the residual bacterial count.

DHP treatment of phosphate buffer

Dynamic high pressure was performed using an Emulsiflex-C5 homogenizer (Avestin, Ottawa). Parameters tested were pressure (100, 200 and 300 MPa) and number of passes (1, 3 and 5). We also tested the combined effect of a 10 minute heat treatment at 25, 45, 55 or 60 °C before DHP treatment at 200 MPa for one pass and the effect of initial bacterial concentration on the DHP treatment (200 MPa /1 pass). 50 ml of phosphate buffer (pH 7.3) was inoculated at a concentration of 10^8-10^9 CFU/ml. The sample was then treated at dynamic high pressure under different conditions. An enumeration for each bacterial strain was made on TSA (Difco) to determine the number of CFU for each treated sample. A serial dilution was made in phosphate buffer and 20 µL was plated on TSA. The phosphate buffer samples were observed by electron microscopy for each treatment (100, 200 and 300 MPa) to visualise the effect of high pressure on bacterial cells.

DHP treatment of raw milk

Fresh raw milk was obtained from Natrel (Quebec city, Can.) the day of the experiment and divided into 50-ml samples. Each sample was then inoculated with different concentrations of *L. monocytogeneses* or *E. coli* and submitted to a DHP treatment as described above. Residual bacteria were enumerated on selective medium. Oxford medium base use with Bacto Modified Oxford Antimicrobic Supplement (Difco) was used for

enumerating *L. monocytogeneses* and MacConkey Sorbitol Agar (Difco) was used for *E. coli*. Results were expressed as N/N_o.

Industrial trial

A pilot-scale test was performed at Avestin Inc. in Ottawa to evaluate the efficiency of the industrial device. Dynamic high-pressure was performed using an Emulsiflex-C160 homogenizer (Avestin, Ottawa) with a flow rate of 160 L/h. For this purpose, a raw milk sample (800 ml) was inoculated with *L. innocua* at 10^7 CFU/ml and submitted to a DHP treatment at a pressure of 200 MPa with 1, 3 and 5 passes. The efficiency of the treatment applied was evaluated by enumerating the residual *L. innocua* in modified Oxford medium and by calculating the N/No ratio. Results were compared to those obtained in the laboratory using the Emulsiflex-C5.

RESULTS

Phosphate buffer results: Fig. 1 illustrates the effect of dynamic high pressure treatment at different pressure (100, 200 and 300 MPa) on three different strains (Panel A : *Salmonella enteritidis;* Panel B : *Listeria monocytogeneses;* Panel C : *Escherichia coli*. ■ :1 pass; ■ :3 passes; □ :5 passes; □ :HHP). In general, Gram (+) bacteria (*L. monocytogeneses*) are more resistant to high pressure than Gram (-) bacteria. For *L. monocytogeneses*, a DHP of 300 MPa with 3 successive passes was needed to achieve a total reduction (8 log), compared to *E. coli* or *S. enteritidis* that were completely inhibited at 200 MPa after 3 passes. The resistance of *L. monocytogeneses* to DHP is probably due to its wall structure, which is made up of a large number of peptidoglycan layers.

This wall composition imparts to the cell a higher resistance to physical phenomena such as chisel effect, turbulence and cavitation undergone by cells in the homogenizer chamber. Gram (-) cells do not have this characteristic and are less resistant. Most of the dead bacteria show a rupture of the cell envelope due to the DHP treatment. For other bacteria, death resulted from total release of the intracellular material without the rupture of the cell envelope.

Previous research on HHP has shown that pressures between 450-500 MPa lasting 10 to 15 minutes are necessary to obtain a reduction of 7 to 8 log units for *L. innocua* (Gervilla, 1997). Rosella Liberti used 600 MPa of static pressure for 10 minutes to get a 5 log reduction from 10^7 to 10^2 CFU/ml with *L. monocytogeneses*. Similar results with *L. monocytogeneses* were obtained after 3 passes under a pressure of 300 MPa in dynamic pressure. DHP was thus more effective than HHP.

Generally, we observe that the more pressure increases, the higher is the death rate. This fact is more evident in panel B with *L. monocytogeneses*. At 100 MPa, the death rate is very low to compared with 300 MPa. The pressure required to eliminate bacteria depends on temperature, pH, chemical composition of the sample and other factors. The number of passes is also a major factor affecting bacterial concentration.

The effectiveness of DHP appears to be affected by the initial temperature of the sample (Fig. 2). An increase in sample temperature prior to DHP treatment results in a better inactivation rate especially for *Salmonella* and *Listeria*. However, no such effect was observed with *E. coli*. For *Salmonella*, heating the

sample to 55°C for 10 minutes results in an additional 4 log reduction after DHP treatment. Two and one additional log reductions were also obtained for 45°C and 25°C respectively. For *Listeria*, only 1.5 additional log reduction was obtained when the sample was heated to 55°C for 10 minutes prior to DHP treatment compared to unheated samples. Heat likely weakens the cell membrane hydrogen and hydrophobic bonds and the bacteria consequently become less resistant to high pressure.

The impact of initial load on the DHP treatment (200 MPa/1pass) is shown in Fig. 3. In general, best inactivation rates were obtained with the lowest bacterial concentration. Once again, *L. monocytogenes* was shown to be the more resistant bacteria compared to the other strains. For *Listeria*, a total inactivation effect was obtained at a concentration of 10^4 CFU/ml while the same effect was obtained at 10^6 and 10^7 CFU/ml for *S. enteritidis* and *E. coli* respectively.

Raw milk results: Two pathogens were tested in milk samples, *L. monocytogenese* and *E. coli*. The effect of pressure and number of passes is shown in Fig. 4 (Panel A : *Listeria monocytogeneses;* Panel B : *Escherichia coli.* ■ :1 pass; ■ :3 passes; □ :5 passes). The reduction of viable bacteria is generally a little more then 2 log smaller than that obtained in phosphate buffer experiments. At 200 MPa (5 passes), a 5.3 log reduction was obtained in the phosphate buffer, whereas in raw milk, only 2.6 reduction was obtained for *L. monocytogeneses*. This phenomenon is even more evident under 300 MPa pressure with 8.3 log and 5.6 log for phosphate buffer and milk respectively.

This difference can be related to the fact that some milk elements such as proteins and fat should have a protective effect on bacteria. The bacteria were fixed to the fat globules and when the sample was homogenized, these globules reduce the effect of physical phenomena such as cavitation, chisel effect and turbulence on the bacteria. This effect was less evident at low pressures. Starting with a microbial concentration of 10^8 CFU/ml, a drop of 1 log was observed even after 5 passes for both the buffer and milk with *L. monocytogeneses*.

The effect of mild heat treatment before homogenization on bacterial reduction in a sample of milk is shown in Fig. 5 (▨ *Escherichia coli;* ■: *Listeria monocytogeneses*). The tested temperatures were 25, 45, 55 and 60°C and the pressure was maintained at 200 MPa for only one pass. We observed that the effect was minor at the lower temperatures (25 and 45 °C) but considerable at the higher temperatures (55 and 60°C). With heating at 60 °C, we obtained a difference of 1.1 log for *E. coli* and 1.5 log for *L. monocytogenese* compared to 55 °C which we attribute to the same membrane effects as in phosphate buffer.

The impact of initial load on the DHP treatment (200 MPa/1pass) milk is shown in Fig. 6. (▨ *Escherichia coli;* ■*:Listeria monocytogeneses*). Contrary to the buffer result, we noted no effects on bacterial viability. We explain this result by the protective effect of milk. For each concentration, the effect is the same on the bacteria. This may be due to fat globules binding to the bacteria and protecting them.

Finally, Fig. 7 shows the industrial trial compared to laboratory results for Listeria innocua under the same treatment conditions as above. A similar reduction was obtained (□ :1 pass; □ :3 passes; □ :5 passes).

This study has shown the effectiveness of DHP for destroying pathogenic flora in milk. It has been shown to be a viable alternative to conventional milk pasteurisation. A better bactericidal effect was obtained compared to hydrostatic pressure and milk characteristics were not affected. This new technology should be given serious consideration in the milk industry.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A process for continuously reducing presence of microorganisms in liquid food product without denaturation comprising the steps of:

a) pressurizing a liquid food product;

b) passing said liquid food product to be treated through a continuous pressurizing circulating system at a non-denaturing temperature comprising a dynamic high pressure homogenizer; and

c) collecting said liquid food product containing a reduced presence of microbes.

2. The process according to claim 1, wherein said pressure of step a) is between about 50 MPa to 500 MPa.

3. The process according to claim 1, wherein said passage of step b) is at least one passage of said liquid food product through the dynamic high pressure homogenizer.

4. The process according to claim 1, wherein said microorganisms are selected from the group consisting of bacteria, fungi, mould, bacteriophage, protozoan, and virus.

5. The process according to claim 1, wherein said temperature is between about 4°C to 55°C.

6. The process according to claim 1, wherein said homogenizer is a high-pressure homogenizer.

7. The process according to claim 1, wherein said liquid food product is selected from the group consisting of milk, juice, liquid food fat, oil, and water.

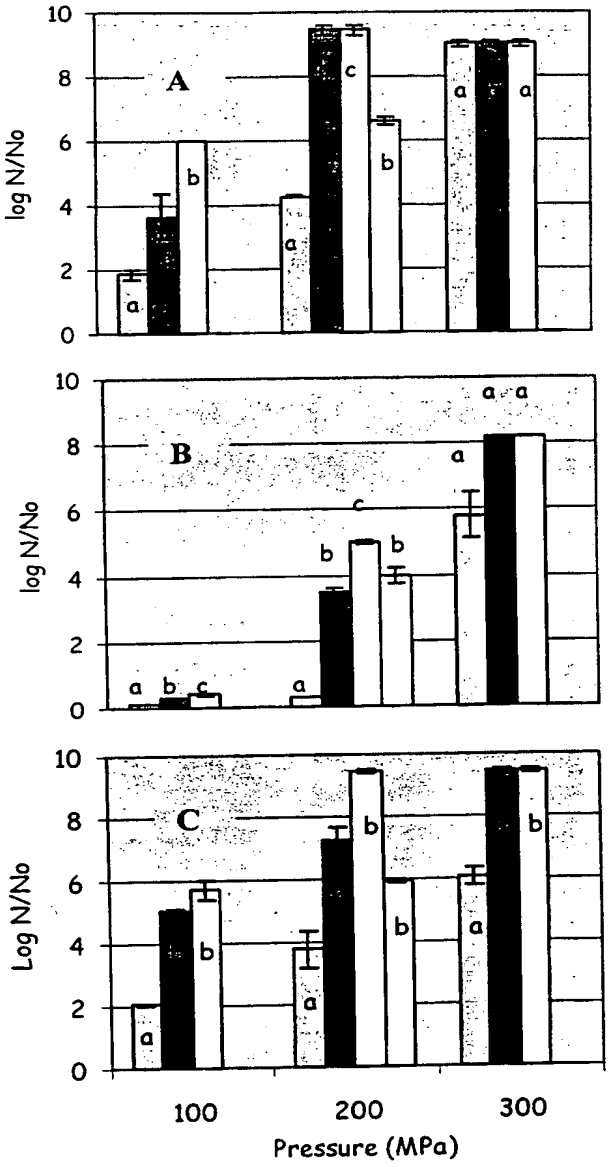


Fig. 1

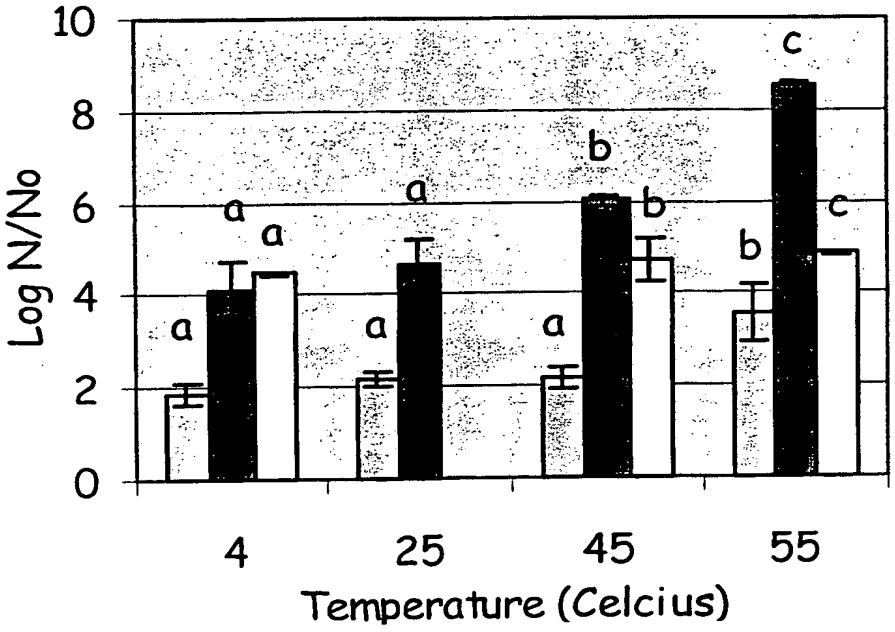
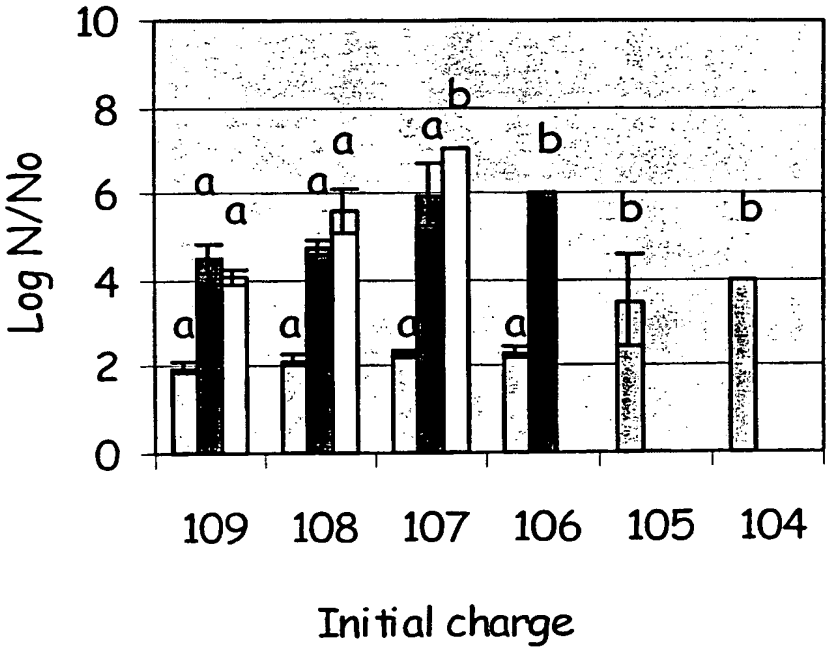


Fig. 2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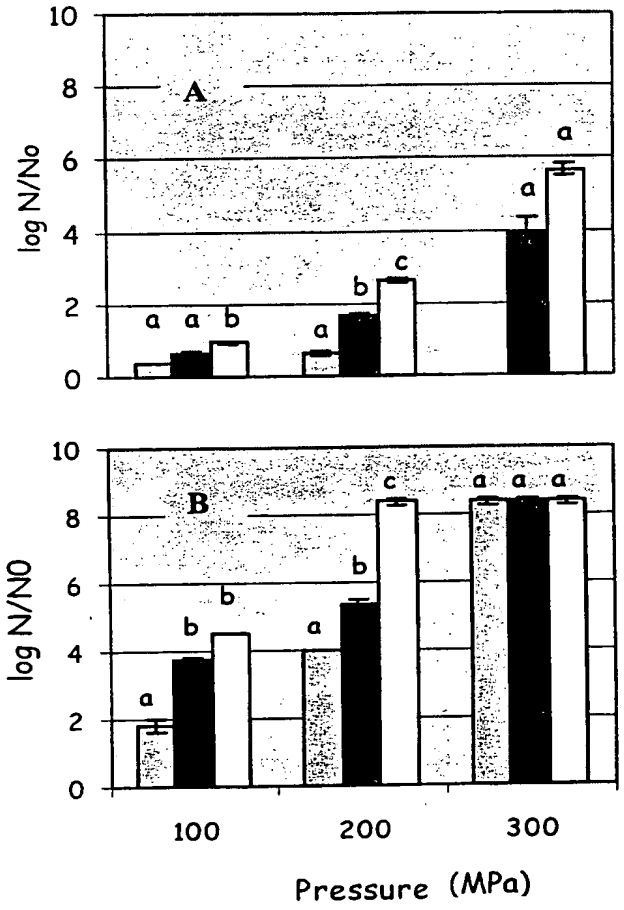
8
6
4
2
0
Log N/No
a
a
a
a
a
a
a
b
a
b
b
b
109
108
107
106
105
104
Initial charge

Fig. 3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B
log N/No
log N/N0
10
8
6
4
2
0
a a b
a b c
a a
a b b
a b c
a a a
100
200
300
Pressure (MPa)

Fig. 4

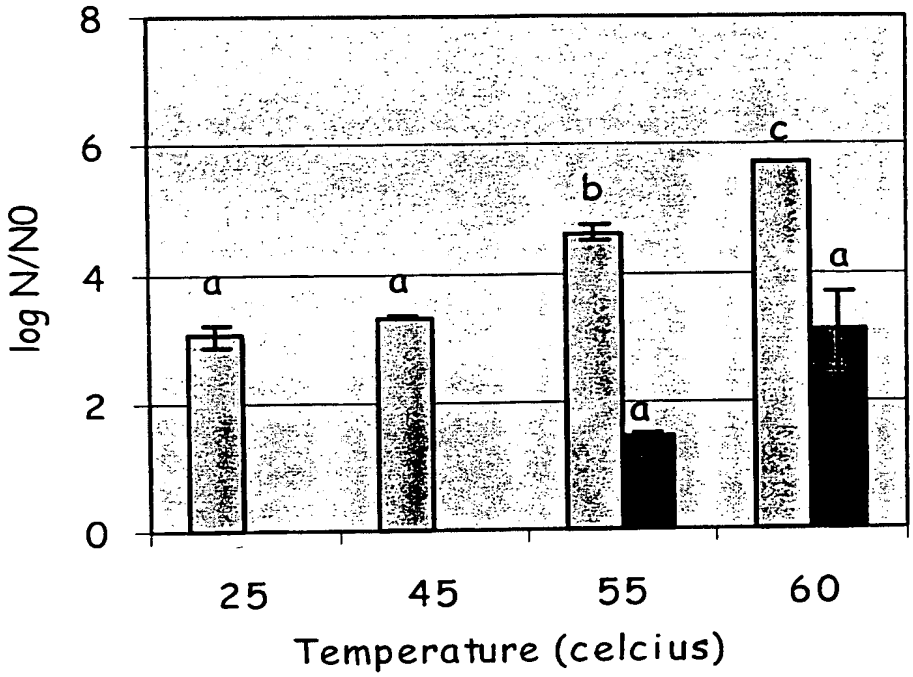

Fig. 5

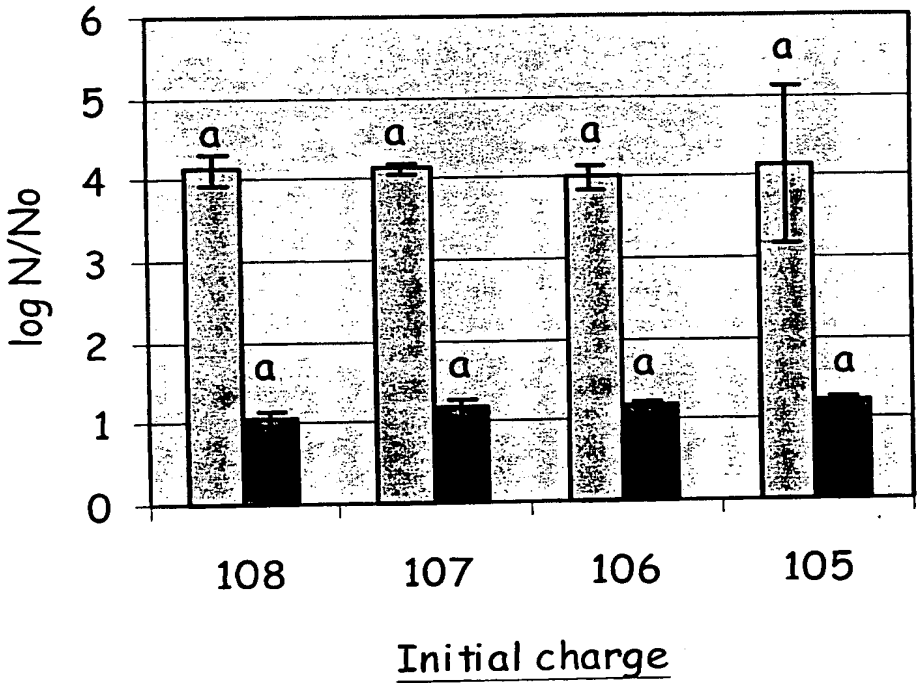

Fig. 6

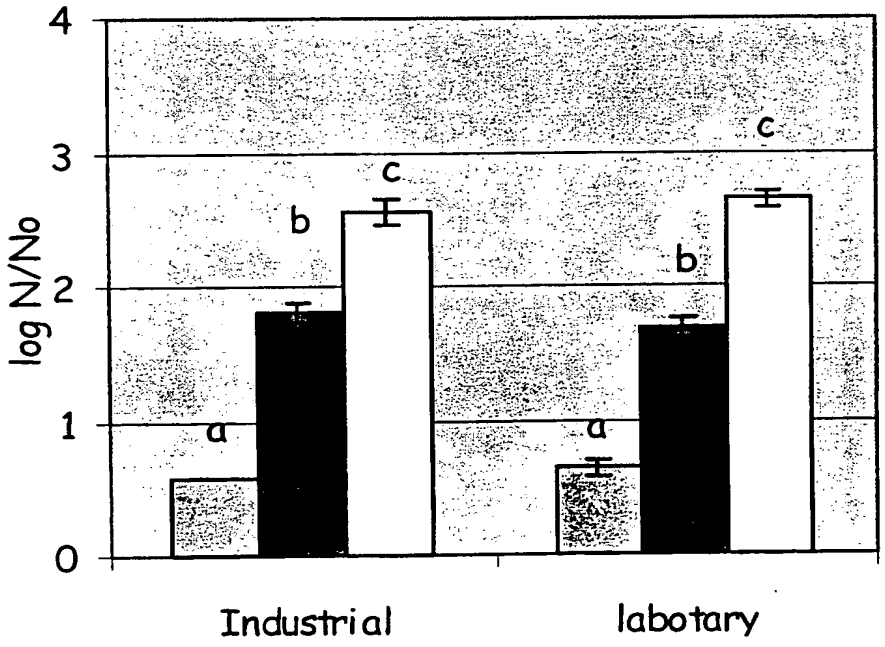

Fig. 7

INTERNATIONAL SEARCH REPORT

Int[illegible]nal Application No
PC[illegible]A 00/00621

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L3/015

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L A23C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
PAJ, EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 September 2000	04/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Guyon, R

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Int [redacted]nal Application No
PC[redacted]A 00/00621

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